ENZYMATIC-ENHANCED PRODUCTION OF GAHARU OIL: EFFECTS OF SHAKING SPEED AND WATER /GAHARU RATIO

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SUPERVISOR'S DECLARATION

"I hereby declare that I had read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the purpose of the granting of Bachelor of Chemical and Natural Resources Engineering."

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I declare that this thesis entitled "*Enzymatic-Enhanced Production of Gaharu Oil : Effects of Shaking Speed and Water/gaharu Ratio*" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree

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DEDICATION

Special dedication to my beloved father,mother, brothers,sister

&

Zaharatun Nadwa Binti Sha'rani

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ABSTRACT

Gaharu with scientific named Aquilaria is a very valuable plant where widely used for medicine, perfumery, and incense. Due to its rarity and the high growing demand for it, gaharu essential oil brings very high prices. One of the common methods to extract gaharu oil is by hydro distillation. However, the yield of gaharu oil using this method is still in small percentage. Previous researches proved that enzymatic hydrolysis during pre-treatment can give better result of gaharu oil yield extraction. Since enzymes are generally active over a specific range of reaction condition, research to improve the enzymatic hydrolysis has been conducted. Two parameters which are shaking speed (rpm) and water/gaharu ratio (v/w) that affect the enzymatic hydrolysis were studied. From the results, the yields for gaharu oil extraction were increased as the shaking speed increased from 50 rpm to 150 rpm. At the shaking speed of 200 rpm, the yield did not continue the same trend as the yield was decreased. The highest yield for varying rpm is 0.1092 at the 150 rpm. The yields for gaharu oil extraction were decreased due to the increasing water/gaharu ratio from 8:1 v/w to 20:1 v/w. The highest yield for varying water/gaharu ratio is 0.1092 at the ratio of 8: 1 v/w. Based on results obtained, the combination of 150 rpm and water/gaharu ratio of 8: 1 v/w during enzymatic hydrolysis pretreatment produced maximum gaharu oil yield extraction which is 0.1092%.

ABSTRAK

Gaharu dengan nama saintifik Aquilaria adalah tumbuhan yang sangat bernilai di mana sering digunakan untuk perubatan, minyak wangi dan colok. Disebabkan keistimewaannya dan pertumbuhan permintaan yang tinggi, harga minyak gaharu menjadi amat mahal. Salah satu kaedah yang paling biasa digunakan untuk mengekstrak minyak gaharu adalah penyulingan air. Namun, peratusan penghasilan minyak gaharu menggunakan kaedah ini masih di peratusan yang rendah. Kajian-kajian terdahulu telah membuktikan bahawa hidrolisis dengan enzim sebagai pra-rawatan boleh memberikan hasil pengekstrakan minyak gaharu yang lebih baik dengan peratus penghasilan lebih tinggi. Oleh kerana enzim aktif pada had keadaan tindak balas yang khusus, kajian untuk mengkaji hidrolisis dengan enzim telah dijalankan. Dua parameter iaitu kelajuan goncangan dan nisbah air gaharu yang memberikan kesan kepada proses hidrolisis menggunakan enzim telah dikaji. Parameter eksperimen yang optimum boleh digunakan untuk meningkatkan penghasilan minyak gaharu. Daripada eksperimen, penghasilan minyak gaharu meningkat apabile kelajuan goncangan meningkat dari 50 rpm kepada 150 rpm. Pada kelajuan mengoncang 200 rpm, penghasilan minyak tidak mengikut corak yang sama di mana penghasilan minyak adalah menurun. Peratus penghasilan yang tertinggi untuk kepelbagaian kelajuan mengoncang adalah 0.1092 % pada 150 rpm. Penghasilan minyak gaharu berkadar langsung berkurangan apabila pecahan air per gaharu meningkat dari 8:1 v/w kepada 20:1 v/w. Peratusan penghasilan minyak yang tertinggi untuk kepelbagaian nisbah air gaharu adalah 0.1092 % pada nisbah 8:1. Daripada keputusan, kelajuan goncangan 150 rpm dan pecahan air per gaharu 8:1 semasa hidrolisis dengan enzim menghasilkan peratus penghasilan ekstrak minyak gaharu yang maksimum iaitu sebanyak 0.1092%.

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LIST OF SYMBOLS

RPM	-	rotation per minute
v	-	volume
w	-	weight
h	-	hour
g	-	gram
mL	-	milliliter
L	-	liter
M	-	mole

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Gaharu with scientific named Aquilaria is classified under the family of *Thymelaeaceae*. Aquilaria is the only one tropical tree genus which currently has been focused by international attention (Chakrabarty *et al*., 1994; Ng *et al*., 1997). There are other's name for this incense wood given by local and the world like agaru, aloes wood, agarwood, oud, chen-xiang, eagle wood, jinkoh and others (Cheksum *et al*., 2002). The fragrance of agarwood can vary greatly depending on the country of origin, the density of resin and on the part of the tree from which it is harvested. Gaharu essential oil is very valuable which widely used for medicine properties, perfumery, and incense (Okugawa *et al*., 1993).

Aquilaria trees are now protected in most countries and the collection of agarwood is illegal from natural forests. International agreements, such as the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora), accepted by 169 countries, is designed to ensure trade in agarwood products from wild trees does not threaten the survival of Aquilaria. Despite these efforts agarwood products from illegally cut trees continues to be sold and unknowing consumers create a demand that helps to destroy the last old growth Aquilaria trees in existence (Blanchette, 2006).

According to Leo Sunari, head of the World Wildlife Fund (WWF) Gaharu project, gaharu is known throughout many Asian countries and commonly use from Asia to the Middle East. It is believed that only Aquilaria trees which are older than 25 years can produce high-grade gaharu, valuable, dark brown- or black-colored heartwood with a very strong smell. From India to Indonesia, market demand for this forest product is very strong and far greater than the supply.

As gaharu essential oil is in high demand today, the research to enhance the gaharu oil production is still dwindling. The small percentage of gaharu essential oil production is not enough to meet the market demand. Due to its rarity and the high growing demand for it, gaharu essential oil brings high prices.

Since gaharu is valuable, local entrepreneur has adopted water distillation technique that very much practice traditionally especially in rural areas of Cambodia and India (Chang *et al*., 2002). Nowadays, local entrepreneur prefer an effective technique that produce higher yield of gaharu oil which is using hydro distillation. It is reported that the proportion of mostly essential oil extracted by hydro distillation 93% and the remaining 7% is extracted by other method such as solvent extraction and CO_2 extraction. Hydro distillation method is believed to produces pure quality of gaharu essential oil because it only uses water rather than other method that use solvent such as solvent extraction. The partition between the water and oil phases of distillation make the separation of the oil is easy and more economical (Masango, 2001).

The gaharu oil production using hydro distillation can be improved if an enzymatic hydrolysis treatment is employed prior to extraction step (Fullbrook, 1983; Marek *et al*., 1990; Tano-Debrah *et al*., 1996). The cell wall degradation caused by the enzyme increases the permeability to the oil through membrane. The use of several enzymes as cellulases, hemicellulases, and amylases has been reported (Lanzani *et al*., 1975; Bhatnagar and Johari, 1987).

Like others plant, gaharu oil is found inside plant cells, linked with wide variety of carbohydrates. In order to facilitate its extraction from the plant cells, it is necessary to degrade the cells walls to increase the permeability for oil. Most carbohydrates in gaharu cell wall are in the form of lignocellulose in which is made up of mainly cellulose, hemicellulose, pectin, and lignin. Lignocellulose is generally found in the stems, leaves, hulls, husks, and cobs of plants or leaves, branches, and wood of trees. One technique to hydrolyze this lignocellulose contents is by enzymatic hydrolysis. Degradation of lignocellulose, specifically cellulose by enzyme during enzymatic hydrolysis will increase the permeability to the gaharu oil through of cell wall.

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes which are highly specific (Beguin and Aubert, 1994). The products of the hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low compared to acid or alkali hydrolysis because enzyme hydrolysis usually operate at mild conditions, pH 4.8 and temperature 45 $^{\circ}$C – 50 $^{\circ}$C and does not have a corrosion problem (Duff and Murray , 1996).

1.2 Problem statement

Upon hydro distillation of Malaysian ‘gaharu’ (agarwood), an essential oil is obtained in 0.8% yield (Yaacob, 1999). This show that the yield of gaharu essential oil using hydro distillation still in small percentage compared with mostly types of essential oil extracted by the same method which is 93%. However the drawback of this method is that the still could get overheated, thus burning the aromatics and resulting in a burnt smell (Aromacures, 2006).

The results from previous research indicates that yield of extraction gaharu essential oil using enzyme as pre-treatment (enzymatic hydrolysis) give the highest result compare to extraction without enzyme pre-treatment. The oil production can be improved if an enzymatic treatment is applied (Fullbrook, 1983; Marek *et al*., 1990; Tano-Debrah *et al*., 1996).

The advantages of this biological pretreatment (enzymatic pretreatment) include low energy requirement and mild environmental conditions. However, the rate of most hydrolysis in most biological pretreatment processes is very low (Sun and Cheng, 2002).

There are many factors that effects the enzymatic hydrolysis of cellulose in gaharu wood include substrates, cellulose activity, and reaction condition (pH, temperature, as well as other parameter). Enzymes are generally active over a specific range of reaction condition. Hence, to improve the yield and rate of the enzymatic hydrolysis, research has focused on optimizing the hydrolysis process and enhancing cellulose activity (Cantwell *et al*., 1988; Durand *et al*., 1988; Orpin, 1988).

This research investigated the effects of the specific parameters which are shaking speed (rpm) and water/gaharu (v/w) ratio in enzymatic hydrolysis using cellulase enzyme to enhance production of gaharu oil. As a result, the best parameter of reaction condition during enzymatic hydrolysis can be determined to produce maximum yield of the gaharu oil.

1.3 Scope

Use enzymatic hydrolysis during pre treatment in order to enhance the production of oil from gaharu wood. Further oil extraction process has been conducted via hydro distillation. The effects of shaking speed (rpm) and water/gaharu ratio (v/w) on gaharu oil extraction in enzymatic pre treatment has been investigated.

1.4 Objectives

The objectives of this research are:

- To study the effect of shaking speed (rpm) in enzymatic hydrolysis pre-treatment to get the maximum gaharu essential oil production.
- To study the effect of water/gaharu ratio (v/w) in enzymatic hydrolysis pre-treatment to get the maximum gaharu essential oil production.

CHAPTER 2

LITERATURE REVIEW

2.1 Gaharu

There are a few names for the resinous, fragrant and highly valuable heartwood produced by Aquilaria tree which are agarwood, eaglewood, gaharu and aloeswood. Most common name that use in scientific journal is agarwood. In Malaysia, this incense wood is familiar with name of gaharu (Cheksum *et al*, 2002).

2.1.1 Aquilaria species

Around the tropical region there has been reported that 15 species of Aquilaria exist in India, Burma, China, Myanmar and Malaysia region. In Malaysia there are 5 species of Aquilaria found which are *Aquilaria Hirta*, *Aquilaria Malaccensis*, *Aquilaria Rostrata*, *Aquilaria Microcorpa* and *Aquilaria Becanana*. A significant number of research studies have been conducted on *Aquilaria malaccensis* (Ng *et al*., 1997) which is well distributed throughout Peninsular Malysia except for Kedah and Perlis. *Aquilaria malaccensis* also considered threatened species due to its high value in today's market and has been included in 'The World List of Threatened Tress' (Oldfield *et al*., 1998).

2.1.2 Aquilaria tree and agarwood production

The Aquilaria tree is a large evergreen tree growing over 15-30 m tall and 1.5 – 2.5 m in diameter with white flowers (Chakrabarty *et al*., 1994). According to Professor Robert Blanchette from The University of Minnesota; agarwood is formed when aquilaria trees produce a resin as a defense mechanism against fungi infection or injury causing its normally soft, white wood to become hard and dark in color. This resin-soaked wood is called agarwood (Blanchette, 2006). Agarwood produced in grown Aquilaria tree is shown in Figure 2.0.

Figure 2.0: Agarwood produced in grown Aquilaria tree

The formation of agarwood occurs in the trunk and roots of trees are due to the infection by a parasitc ascomycetous mould, *Phialophora parasitica*, a dematiaceous (dark-walled) fungus. As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding fungal growth. While the unaffected wood of the tree is relatively light in colour, the resin dramatically increases the mass and density of the affected wood, changing its colour from pale beige to dark brown or black.

High quality resin comes from a tree's natural immune response to a fungal attack. It is commonly known as agarwood #1 (first quality). An inferior resin is created using a forced method where aquilaria trees are deliberately wounded, leaving them more susceptible to a fungal attack. This is commonly called as agarwood #2 which the second quality of agarwood (Ng *et al*., 1997).

2.1.3 Gaharu in Malaysia

Today, gaharu or agarwood is becoming more popular in Malaysia. This is due to an initiative taken by En Sulaiman Bin Doss Mohammed Khan, from Muar, Johor to create awareness of the precious sources of agarwood in Malaysia. This awareness is vital as Malaysia is rich in gaharu, mainly in Terengganu and Pahang. The Malaysia government recently financed some agencies to continue research and development of gaharu. The goal is also to increase the trade of agarwood in Malaysia (Ng *et al*., 1997).

Since price of gaharu is very high where the good quality gaharu can fetch around RM10, 000 per kg, gaharu collectors or buyers have to pay a royalty fee amounting to 10% of the raw material market price. Convention on International Trade in Endangered Species (CITES) have been issued an extraction permit and facilitate the traders in obtaining export of gaharu wood (Hilary, 2005).

2.1.4 Uses of gaharu

2.1.4.1 Medicine

Agarwood is one of the earliest recorded medicines found in early Chinese medical textbooks. The main function of agarwood is to remove the bad chi or energy from the body, which promotes circulation and blood flow. High grade agarwood powder is prescribed in Chinese medicine and used in the production of pharmaceutical tinctures (Yaacob, 1999).

Agarwood is used as a complex ointment for smallpox and various abdominal complaints. It also prescribed for dropsy, as a carminative, for heart palpitations and as a tonic taken particularly during pregnancy, after childbirth and disease of female genital organs (Chakrabarty *et al*., 1994).

2.1.4.2 Perfume

Perfume is a mixture of fragrant essential oils and aroma compounds, fixatives, and solvents used to give the human body, objects, and living spaces a pleasant smell. Agarwood is said to have been highly prized by European perfumes in the mid – 1990s (Chakrabarty *et al*., 1994).

In India, various grade of agarwood distilled separately before blending to produce final “minyak attar”. Minyak attar is water-based perfume containing agarwood oil which is traditionally used by Muslims to lace prayer clothes (Yaacob, 1999). Agarwood perfumes are seldom pure agarwood oil, but instead use an alcoholic or non alcoholic carrier. The cheapest agarwood perfumes are either synthetic or a blend of oils each with different qualities and fragrances. Agarwood essences have recently been used as fragrances in soaps and shampoo (Chakrabarty *et al*., 1994).

2.1.4.3 Incense

Agarwood powder and dust cannot be burned directly in incense holders, but can be used to make incense sticks or coils for indoor fragnance. Agarwood incense is burned to produce a pleasant aromatherapy. The aromatic smell of this incense is 100 % pure natural smell that is the specific smell of each different area of Agarwood. No chemicals or any artificial perfumes added. So they are very safe and no side effects to human health when burning. The most distinguishable advantage of Agarwood Incense is that it can be used in closed environments (Agarwood incense, 2007).

Pure agarwood is also burned as incense in Japan. The agarwood is breaks pieces off and burn. A revival in the ancient art of *Koh doh* (incense ceremony) in Japan has revitalized interest in agarwood (Katz, 1996).

2.2 Extraction

2.2.1 Definition of extraction

Extraction is a separation process to separate solute or removed undesirable solute component from the solid where the solid is contacted with a liquid phase. Fragrance extraction are processes which involve extracting aromatic compounds from the raw material using various methods such as distillation, solvent extraction and expression Currently, the most popular method that used many old times for essential oil extraction is distillation (Gilbert and Martin, 2002).

2.2.2 Distillation

Distillation accounts for the major share of essential oils being produced today. The choice of a particular process for the extraction of essential oil is generally dictated by the following: (1) sensitivity of the essential oils to the action of heat and water; (2) volatility of the essential oil; and (3) water solubility of the essential oil (Hand Book on Medicinal, 2007). After extraction, the properties of a good quality essential oil should be as close as possible to the essence of the original plant. The key to a good essential oil is through low pressure and low temperature processing. High temperatures, rapid processing and the use of solvents alter the molecular structure, will destroy the therapeutic value and alter the fragrance. This cause the usually method choose for oil extraction is hydro distillation.

2.2.3 Hydro distillation

Hydro distillation is one of the oldest and easiest methods being used for the extraction of essential oils using the water. It is not only the most ancient method of distillation but also the most versatile. In this method the plant material is fully dipped and boiled in the water with the resultant steam being captured and condensed. It involves the use of a common tub to boil water and plant material. Hydro distillation is the best method for tough materials like nuts, wood or root. However the disadvantage of this method is that the still could get overheated, thus burning the aromatics and resulting in a burnt smell (Aromacures, 2006).

2.3 Enzymatic hydrolysis

2.3.1 Cellulase

Enzymatic hydrolysis of cellulose is carried out by cellulase enzyme which is highly specified (Beguin and Aubert, 1994). Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic material. These organism can be aerobic or anaerobic, mesophilic or thermophilic.

Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase which attacks regions of low crystallinity in the cellulose fiber, creating free chain-end; (2) exoglucanase or cellobiohydrolase, which degrades the molecule further by removing cellobiose units from the free chain-ends; (3) ß- glucosidase which hydrolyzes cellobiose to produce glucose. In addition to the three major groups of cellulose enzymes, there are also a number of ancillary enzymes that attack hemicellulose such as glucuronidase, xylanase, galactomannanase and glucomannanase (Duff and Murray, 1996).